

Persistence of Infectious HIV in Both Memory and Naive CD4+ T Cell Subsets in Patients on Prolonged and Effective HAART

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Abstract

Background: In patients on prolonged effective HAART, it has been demonstrated the persistence of infected CD4+ T cells that harbor latent replication-competent virus. To investigate the phenotypic features of these infected lymphocytes, we examined four highly purified subsets of memory and naive CD4+ T cells for the presence of replication-competent virus.

Methods: In 11 highly adherent patients on long-term HAART, with undetectable plasma virus, we isolated by using a magnetic bead-based procedure highly purified CD4⁺CD45RO⁺, CD4⁺CD45RA⁺ T cells. Then a subsequent cell separation according to CD62L expression was carried out. We quantified total viral DNA in freshly isolated T cell subsets by means of quantitative PCR. To verify whether HIV DNA was competent for replication, HIV RNA was measured in supernatants following 2 days of cell activation.

Results: HIV DNA was detectable in the CD45RO⁺CD4⁺ and CD45RA⁺CD4⁺ T cell subsets in 100% and 90% of the patients tested. In central memory CD45RO⁺CD62L⁺, effector CD45RO⁺CD62L⁻, truly naive CD45RA⁺CD62L⁺, and CD45RA⁺CD62L⁻CD4⁺ T cells, HIV DNA was found in 100%, 55%, 88%, and 59% of the patients tested, respectively. HIV DNA amount was significantly higher in the CD45RO⁺ fraction as compared to the CD45RA⁺ subset ($p < 0.01$, Wilcoxon test) and in the CD45RO⁺CD62L⁺ fraction as compared to the three other CD45RO⁺CD62L⁻ subsets ($p < 0.01$). Detectable HIV RNA was found in the culture supernatants of CD45RO⁺CD4⁺ and CD45RA⁺CD4⁺ T cell subsets in 80% and 66% of the patients tested and in CD45RO⁺CD62L⁺, CD45RO⁺CD62L⁻, CD45RA⁺CD62L⁺, and CD45RA⁺CD62L⁻CD4⁺ T cells in 100%, 100%, 100% and 50% of the patients tested.

Conclusions: In patients on prolonged and effective HAART, the pool of infected CD4+ T lymphocytes includes a predominant part of memory cells but also cells of naive phenotype. This should be taken into account in the therapeutic strategies to reduce the pool of HIV-reservoir cells.

Introduction

In patients on prolonged HAART with undetectable plasma viral RNA, the persistence of a stable pool of resting CD4+ T cells that harbor latent replication-competent virus has been demonstrated [1]. It has been hypothesized that this reservoir is derived from activated cells that reverted to a quiescent state [1]. Supporting this hypothesis, replication-competent HIV was detected in CD45RO⁺CD4⁺ memory T cells [1]. However, in patients on prolonged and effective HAART, besides memory CD4+ T cells, other T cell subsets such as naive CD45RA⁺CD62L⁺CD4⁺ T cells may contain infectious HIV, and release virus following HAART interruption. To examine this issue, we investigated the presence of replication-competent virus in naive as well as memory CD4+ T lymphocytes, separated according to CD45RA/CD45RO and CD62L expression.

Results

* We investigated the presence of viral DNA in highly purified CD45RO⁺ and CD45RA⁺CD4⁺ T cell subsets and in the following CD4+ T cell subsets selected according to CD45RO/RA and CD62L expression: CD45RO⁺CD62L⁻, CD45RO⁺CD62L⁺, CD45RA⁺CD62L⁻, CD45RA⁺CD62L⁺ (see Fig 1). As shown in Figure 2, viral DNA was detected in the CD4⁺CD45RO⁺ and CD4⁺CD45RA⁺ subsets respectively in 100% and 90% of all patients. Nine patients had sufficient cell isolation yields to test for the presence of viral DNA in the four CD4⁺CD45RO/CD62L cell subsets. Viral DNA was detected in the CD45RO⁺CD62L⁺ and CD45RO⁺CD62L⁻ subsets in 100% and 55% of patients respectively, and in the CD45RA⁺CD62L⁻ and CD45RA⁺CD62L⁺ subsets in 88% and 50% of cases respectively. We quantified viral DNA in the various CD4+ T cell subsets. As shown in Figure 2, HIV DNA was significantly higher in the CD45RO⁺ fraction (median: 245, range: 22-3563) as compared to the CD45RA⁺ subset (median: 32, range: 0-2166) ($p < 0.01$), and in the CD45RO⁺CD62L⁺ fraction (median: 68, range: 5-3133) as compared to the CD45RA⁺CD62L⁻ subset (median: 16, range: 0-701) ($p < 0.01$) and the CD45RA⁺CD62L⁺ subset (median: 1, range: 0-330) ($p < 0.01$). The difference in HIV DNA amounts between CD45RO⁺CD62L⁺ and CD45RO⁺CD62L⁻ subsets (median: 14, range: 0-1108) was barely significant ($p = 0.055$). Statistical analysis showed no significant difference in terms of HIV DNA amounts between CD45RO⁺CD62L⁻ subsets and either

CD45RA⁺CD62L⁻ or CD45RA⁺CD62L⁺ subsets, $p = 0.31$ and 0.125 respectively. Among CD45RA⁺CD4⁺ T cells, HIV DNA was higher in the CD62L⁺ subset ($p < 0.05$). We also examined viral DNA in highly purified monocytes. Viral DNA was found in 5 of 11 tested patients (45%). HIV DNA in monocytes was lower than in both CD45RO⁺ and CD45RA⁺ T cells (median: 0, range: 0 to 100) ($p < 0.01$).

* To verify whether the HIV DNA detected corresponds to replication-competent virus, whenever the cell isolation yield allowed it, we strongly activated cell subsets positive for HIV DNA. Then HIV RNA was quantified in supernatants (see Fig 3). For the CD45RO⁺CD62L⁻ subsets, cell isolation yields allowed cell activation only in 2 to 4 patients. Infectious virus was found in HIV DNA⁺CD4⁺ T cells in 100% of the cases. Replication-competent virus was found in HIV DNA⁺CD45RO⁺ and CD45RA⁺CD4⁺ T cells in 80% and 66% of the cases respectively (Fig 3). Infectious HIV was detected in HIV DNA⁺ central memory CD45RO⁺CD62L⁻, effector memory CD45RO⁺CD62L⁻, truly naive CD45RA⁺CD62L⁺, and CD45RA⁺CD62L⁻ T cells in 100%, 100%, 100% and 50% of the cases respectively. HIV replication-competent virus was found in HIV DNA⁺ monocytes in 80% of the cases.

Figure 1. Purity of CD45RO/RA CD62L± CD4+ T cell subsets.

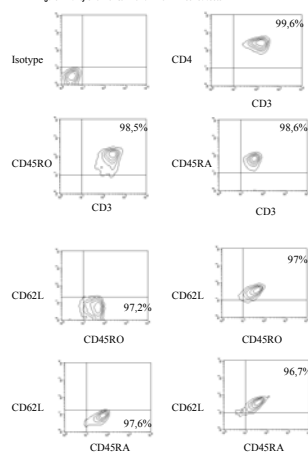


Figure 2. Quantitation of viral DNA in the purified cell subsets.

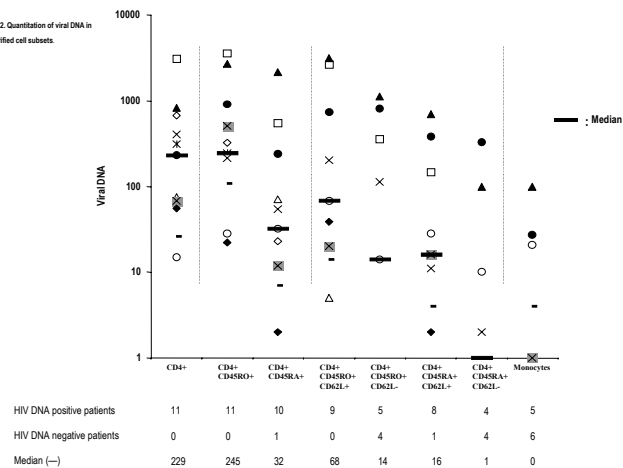
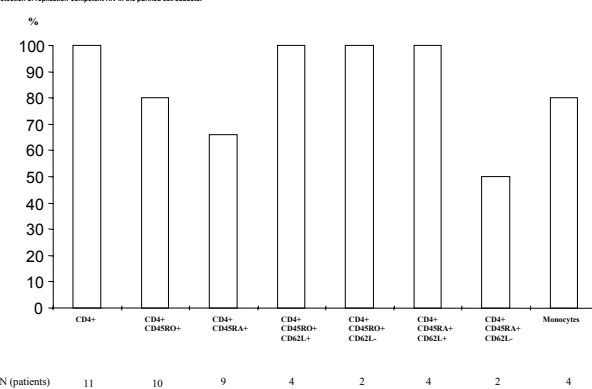


Figure 3. Detection of replication-competent HIV in the purified cell subsets.



Discussion

In highly selected patients on prolonged and effective HAART, we found various levels of HIV DNA in memory CD45RO⁺ and naive CD45RA⁺, and in the four CD45RO/CD45RA/CD62L± CD4+ T cell subsets. CD45RO⁺CD62L⁻, CD45RO⁺CD62L⁺, CD45RA⁺CD62L⁻ cells correspond to central memory, effector memory, and truly naive CD4+ T cells respectively [1]. Total viral DNA was significantly higher in the CD45RO⁺ total subset as compared to the CD45RA⁺ total subset. Two non-exclusive hypotheses may be put forward to explain this result. First, CD45RO⁺CD4⁺ T cells can be infected by both non-synchronizing (NS) and synchronizing (S) variants, while CD45RA⁺CD4⁺ T cells are exclusively infected by SI virus [4]. This may be related to the differential expression of the virus co-receptors CXCR4 and CXCR4 at the cell surface of CD45RO⁺ and CD45RA⁺CD4⁺ T cells. Second, the excess viral DNA in memory CD4+ T cell subsets may be related to integrated virus in the context of [1] that while no integrated viral DNA may be found indifferently in both memory and naive CD4+ T cells, integrated viral DNA is mainly found in memory CD4+ T cells. This is consistent with the finding that resting CD4+ T cells harboring latent integrated virus, which are a major HIV reservoir in patients on prolonged HAART [1], are memory CD45RO⁺ T cells [1]. This reservoir may be predominantly comprised of infected CD45RO⁺CD62L⁺CD4⁺ T cells, as the highest amount of HIV DNA was found in this central memory subset. We detected, in patients on prolonged

HAART, the presence of virus in truly naive CD4+ T cell subset. HIV infection to virus integration of naive cells is controversial [5,6], suggesting that a major part of this viral DNA is labile non-integrated viral DNA resulting from recent infections. The persistence of a residual viral replication has been demonstrated in patients on prolonged HAART [2,7,8]. However, we found the presence of infectious monocytes, a potential marker of ongoing viral replication [2], only in 4 out of 11 patients. Moreover, a recent report demonstrated the presence of provirus in truly naive CD4+ T cells in a heterogeneous group of patients according to viremia and treatment [9]. Accordingly, we cannot absolutely rule out the fact that the naive CD4+ T cell-related HIV DNA virus detected in the patients of our study group may also contain integrated virus, pointing out that naive CD4+ T cells may be a part of the stable HIV reservoir in patients on prolonged HAART. Integrated proviral DNA in naive lymphocytes might result from the presence of latently infected memory lymphocytes harboring integrated DNA which revert to a naive (CD45RA⁺) phenotype [5], and/or the infection of immature doubly positive CD4⁺CD8⁺ thymocytes [10] which further differentiate towards naive lymphocytes. Altogether, our results suggest that, in patients on prolonged and effective HAART, the pool of infected CD4+ T lymphocytes includes a predominant part of memory cells but also cells of naive phenotype. This should be taken into account in the additional immunotherapeutic strategies to reduce the pool of HIV-infected cells.

References

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